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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PC 080301

AUG 18 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: DEET: Review of acute oral toxicity study of pyridostigmine bromide (PB), permethrin (PERM), and DEET in the laboratory rat

Caswell No.	346	**MRID No.**	None
PC Code.	080301	**DP Barcode:**	D207136

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The Health Effects Division has obtained a report on the effects of oral administration (by gavage) of DEET, permethrin, and pyridostigmine bromide from the U.S. Army Medical Research and Materiel Command. The Study has been reviewed, and the DER is attached. The citation of the study and conclusion of the review are as follows:

Citation: McCain, W.C. (1995) Acute oral toxicity study of pyridostigmine, permethrin, and DEET in the laboratory rat. Unpublished report prepared by U.S. Army Medical Research and Materiel Command. Toxicological Study No. 75-48-2665. May 31, 1995.

Conclusion: In a comparative acute oral toxicity study in male Sprague rats, groups of males (10/dose) received (by gavage) DEET, PERM, or PB at doses ranged from 2000 to 5010 mg/kg for DEET, from 316 to 2000 mg/kg for PERM, and 50 to 126 mg/kg for PB. With probit analysis, **the results indicated that the oral LD_{50} for DEET was 3664 mg/kg; PERM, 1000 mg/kg; PB, 61.36 mg/kg. These LD_{50} values were consistent with existing data.**

In the interaction portion of the study, groups of animals (6 males/dose group) received (by gavage) a single dose which were composed of different combinations of DEET, PERM, and PB. The combinations consisted of varying doses of one chemical (DEET, 0 to 6898 mg/kg; PERM, 0 to 3576 mg/kg; PB, 0 to 83.24 mg/kg) while keeping the other two chemicals at a constant dosage which corresponded to the LD_{16} (DEET, 1946 mg/kg; PERM, 279 mg/kg; PB, 45.76 mg/kg). **The results indicated that combining PB with either PERM or DEET resulted in statistically significant potentiation in mortality which was substantially greater than the expected additive effect (at a dose which corresponds to LD_{16}: 2x for PB + PERM; ≈3x for PB + DEET). In contrast, combining DEET and PERM yielded a mortality rate which was less than the expected additive effect.**

DER #19

DEET: Acute Oral Toxicity Study in Rats
U.S. Army. 1995. MRID No. none. HED Doc. No. 011638.

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Reviewer: Whang Phang, Ph.D. 8/15/95
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DATA EVALUATION REPORT

Study Type: A special study which employs acute oral toxicity parameters to determine the effects of combining pyridostigmine bromide (PB), DEET, and permethrin (PERM)

Chemical: DEET (N, N-diethyl-m-toluamide)

Caswell No. 346 **DP Barcode Code:** 217374
PC Code: 080301 **Submission No.:** This is not a submission.

Sponsor: U.S. Army

Testing Facility: U.S. Army Center for Health Promotion and Preventive Medicine
APG, MD

Citation: McCain, W.C. (1995) Acute oral toxicity study of pyridostigmine, permethrin, and DEET in the laboratory rat. Unpublished report prepared by U.S. Army Medical Research and Materiel Command. Toxicological Study No. 75-48-2665. May 31, 1995.

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The possible mechanisms for the potentiation produced by

combining PB with DEET or PERM are presented in the Discussion section of this DER.

METHODS and MATERIALS

Test article: DEET (technical grade): 98.5% pure; a clear amber liquid with little or no odor. Obtained from Morflex, Inc., Greensboro, NC.

Permethrin (PERM) (technical grade): 91.6% pure with a cis isomer percentage of 42.3%; an amber liquid with a pungent odor. Obtained from Coulston Products, Easton, PA.

Pyridostigmine bromide (BP) is a white crystalline powder with a purity of 99.1%. Obtained from Walter Reed Army Institute of Research, Washington, DC.

Test animals: Male Sprague-Dawley rats (weighing between 250 and 300 gm. These rats were obtained from Charles River, Inc., Raleigh, NC.

STUDY DESIGN and RESULTS

This study consisted of 2 phases.

Phase I, the test animals were divided into 10 animals per dose group, and each chemical was tested in five dose levels. A vehicle control (propylene glycol) group was also included. The dose levels for each chemical are as follows:

	mg/kg				
DEET	2000	2510	3160	3980	5010
PERM	316	511	794	1260	2000
PB	50	63	79	100	126

The test animals were fasted overnight, and test article was administered in propylene glycol by gavage at volume of 10 ml/kg. The treated animals were observed hourly until the dark cycle. Gross necropsy was performed on all test animals as soon as possible after death. The surviving animals were sacrificed after 14 days.

Results: The mortality for each chemical in different doses are presented in Table 1. Using probit analysis, the lethal dose-response information was derived (Table 2). **The results showed that the oral LD_{50} for DEET was 3664 mg/kg;** PERM, 1000 mg/kg; PB, 61.36 mg/kg. The value for oral LD_{50} (3664 mg/kg) is consistent with those (2100 to 3200 mg/kg) reported in the DEET Registration Standard (December 1980).

Table 1+. Mortality in DEET, PERM, and PB treated male rats.

DEET	Dose (mg/kg)				
	2000	2510	3160	3980	5010
Mortality	1/10	3/10	6/10	4/10	7/10
PERM	Dose (mg/kg)				
	316	511	794	1260	2000
Mortality	0/10	5/10	5/10	6/10	6/10
PB	Dose (mg/kg				
	50	63	79	100	126
Mortality	4/10	3/10	8/10	10/10	10/10

+: Data excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).

Table 2+. Dose levels derived from Phase I data for positive control portion of the study.

Probit (%lethality)	PB mg/kg	PERM mg/kg	DEET mg/kg
4 (16%)	45.76	279	1946
4.5 (30%)	52.59	511	2628
5 **(50%)**	**61.36**	**1000**	**3664**
5.5 (70%)	71.59	1953	5109
6 (84%)	83.28	3576	6896

+: Data excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).

Phase II, this phase of the study was divided into 2 portions:

Positive control portion: 15 groups, 6 males per group

Interaction portion: 18 groups, 10 males per group

Animals in the positive control group received the test

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chemicals in propylene glycol by gavage at the dose levels derived by probit analysis of the Phase I data as indicated in Table 1 for the 3 test chemicals.

For the interaction portion of the study, the amimals received varying dose levels of one chemical in combination with two other chemicals, each of which was at the dose level corresponding to 16% lethality as shown in Tables 1 & 2. The combinations for dosing [expressed as lethal dose (LD)] are presented in Table 3.

Table 3[+]. Study design for the interaction portion

Dose Group	First Cmpd LD	Second Cmpd LD	Third Cmpd LD	Exp. Addi.* Cmpd LD
1	16%	16%	0%	32%
2	16%	16%	16%	48%
3	16%	16%	30%	62%
4	16%	16%	50%	82%
5	16%	16%	70%	>100%
6	16%	16%	84%	>100%

+: Data excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).
*: Expected additive compound LD.

After dosing the test animals were observed hourly until the end of the light cycle. The test animals were sacrificed at the end of 14 day study period. Any test animals that died on study or sacrificed at the end of the study were examined for gross pathological lesions.

Results:

Postive control portion: The report stated that data obtained from the positive control group were not significantly different from Phase I dose-response information. No data were presented for this portion of the study.

Interaction portion: Figures 1, 2, & 3 shows the results of administering a combination of the three chemicals.

Figure 1 (p. 7) shows that the actual mortality (dark stippled bar) in rat, which received varying doses of DEET and a dose of PERM and PB which corresponds to LD_{16}, was substantially greater than the expected mortality at dose level of 2628 mg/kg or below. Even at 0 mg/kg DEET (first dosage), PERM and PB induced substantially greater mortality rate (≈2X) than the expected additive effects

dosage of DEET to 3664 mg/kg or more did not produce a corresponding increase in mortality (Figure 1).

Figure 2 (p.7) shows the results of varying the dosage of PERM while keeping the dose of DEET and PB at LD_{16} level. The response of combining DEET and PB in the presence of 0 mg/kg PERM (first dosage) was dramatically greater (3X) than the expected result. The other combinations gave similar results as in Figure 1.

Figure 3 (p.8) shows that in the presence 0 mg/kg PB, PERM and DEET (first dosage) produced a mortality 2X less than the expected mortality. As soon as PB was introduced into the combination, the mortality was greated than that expected.

Figure 4 (p. 8) summarized the results of the first dosage level as shown in Figures 1, 2, & 3 (i.e., the combination of two chemicals). The results showed that combining PB with either PERM or DEET resulted in statistically significant increases in mortalitly, and the increases were substantially greater than the expected additive effects. In contrast, exposure to a combination of DEET and PERM produced a mortality rate, which was less than the expected additive effect.

DISCUSSION

In a comparative acute oral toxicity study in male Sprague rats, groups of males (10/dose) received (by gavage) DEET, PERM, or PB at doses ranged from 2000 to 5010 mg/kg for DEET, from 316 to 2000 mg/kg for PERM, and 50 to 126 mg/kg for PB. With probit analysis, the results indicated that oral LD_{50} for DEET was 3664 mg/kg; PERM, 1000 mg/kg; PB, 61.36 mg/kg. These LD_{50} values were consistent with existing data.

In the interaction portion of the study, groups of animals (6 males/dose group) received (by gavage) a single dose which was composed of different combinations of DEET, PERM, and PB. The combinations consisted of varying doses of one chemical (DEET, 0 to 6898 mg/kg; PERM, 0 to 3576 mg/kg; PB, 0 to 83.24 mg/kg) while keeping the other two chemicals at a constant dosage which corresponded to the LD_{16} (DEET, 1946 mg/kg; PERM, 279 mg/kg; PB, 45.76 mg/kg). **The results indicated that combining PB with either PERM or DEET resulted in statistically significant potentiation in mortality which was substantially greater than the expected additive effect (at a dose which corresponds to LD_{16}: 2X for PB + PERM; 3X for PB + DEET). In contrast, combining DEET and PERM yielded a mortality rate which was less that the expected additive effect.**

The author of the report offered an explanation for the potentiation of mortality produced by the combination of PB with either DEET or PERM. A possible mechanism for DEET is that when PB is combined with DEET, DEET may facilitate the absorption of PB because (1) PB is poorly absorbed by the gut, but (2) DEET has been used as a transdermal carrier molecule for the delivery of drugs and other agents [Hussain and Ritschel (1988), Reifenrath et al (1984), Windheuser et al. (1982)]. This would lead to an increase in the PB level in the blood. Since the mode of action for PB is inhibition of cholinesterase activity, with higher blood levels of PB the inhibition is increased resulting in higher mortality.

A possible mechanism for the observed potention produced by a combination of PB and PERM is the inhibition of the detoxification system (U.S. Army, Study No. 75-48-2665). It has been shown that hydrolysis of the ester bond in PERM is mediated by non-specific esterases. When an animal was exposed concur-rently to both pyridostigmine and PERM, inhibition of the esterases by PB would lead to decreased hydrolysis and increased residence time of permethrin in the body.

References

Windheuser, J.J., Haslam, J.L., Caldwell, L., and Shaffer, R.D. (1982). The use of N,N-diethyl-m-toluamide to enhance the dermal and transdermal delivery of drugs. *J. Pharmacol. Sci. 71(11):1211-1213.*

Hussain, A.S. and Ritschel, W.A. (1988). Influence dimethylacetamide, N,N-diethyl-m-toluamide, and 1-dodecylazacycloheptan-2-one on ex vivo permeation phosphonoformic acid through rat skin. *Method Fund. Exp. Clin. Pharmacol. 10(11):691-694.*

Reifenrath, W.G., Chellquist, E.M., Shipwash, E.A., Jederberg, W.W., and Krueger, G.G. (1984). Percutaneous penetration in the hairless dogs, weanling pigs and grafted athymic nude mouse: evaluation of models for predicting skin penetration in man. *Br. J. Dermatol. 111(Suppl. 27): 123-135.*

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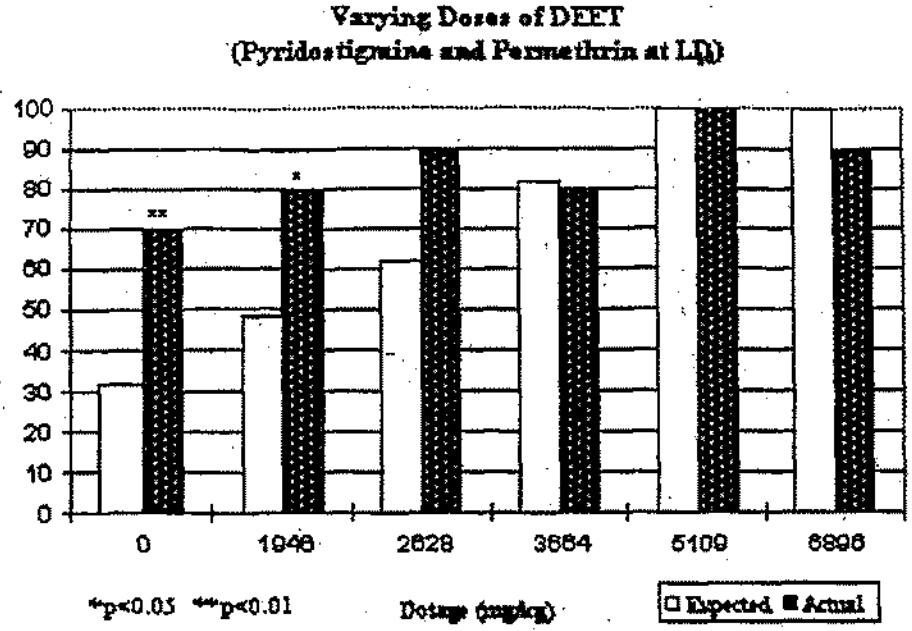

figure 1+. Response of rats given various doses of DEET with LD_{15} of permethrin and pyridostigmine (additive LD_{32}) as compared to expected additive mortality. (n=10 males/group).

+: figure excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).

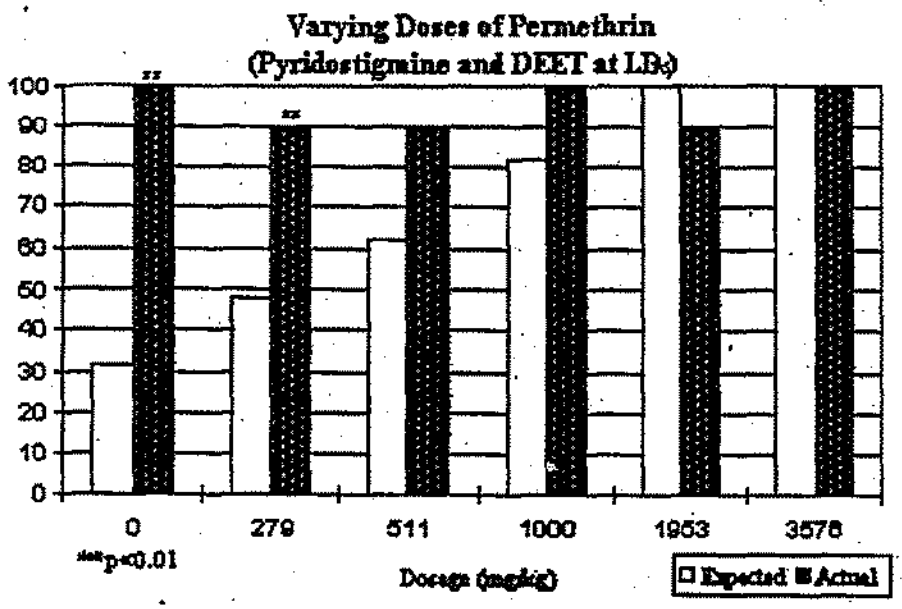

Figure 2+. Response of rats given various doses of permethrin with LD_{15} of DEET and pyridostigmine (additive LD_{32}) as compared to expected additive mortality. (n=10 males/group).

+: Figure excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).

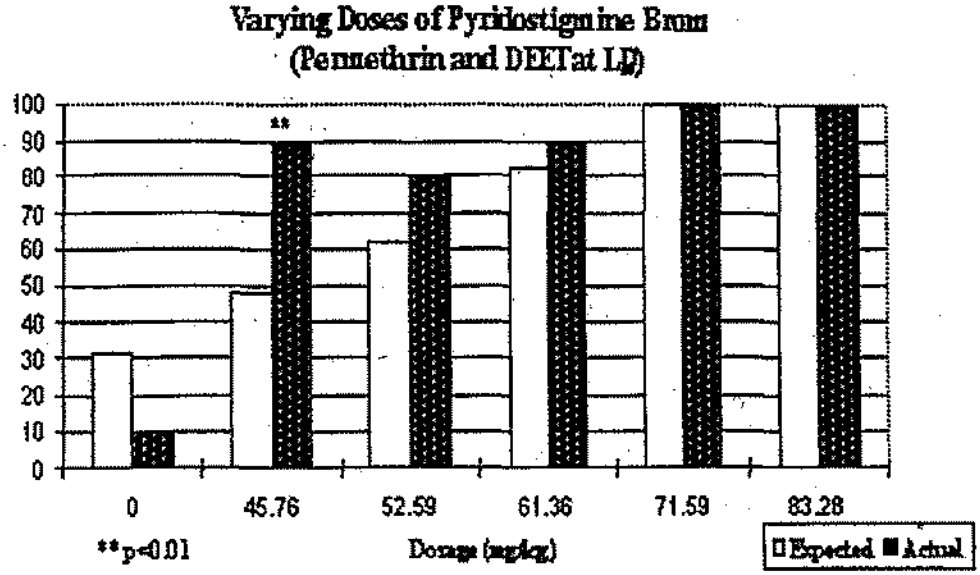

Figure 3+. Response of rats given various doses of pyridostigmine with LD_{16} of permethrin and DEET (additive LD_{32}) as compared to expected additive mortality. (n=10 males/group)

+: Figure excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).

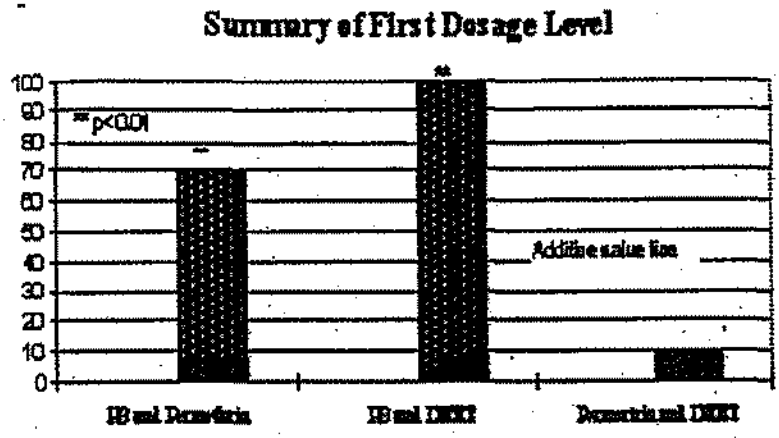

Figure 4+. A Comparison of mortalities associated with the first dosage level which resulted LD_{16} of two compounds (additive LD_{32}) only. (n=10 males/group).

+: Figure excerpted from the U.S. Army, Toxi. Study No. 75-48-2665 (DP217374).